test crosses of plants 6452-3, 6453-3, and 6453-9 deviated very much from 1 : 1 and with plant 6453-9, marked differences in ratio of these two phenotypes were expressed on ears produced ~~from~~ on different parts of this plant. Nevertheless, it was clear that some chromosomal component, present in the a_1^{m-1} carrying plants, and not linked to this locus, was ~~xxxxx~~ segregated at meiosis and that the expression of the uniformly pale class of kernels and the ~~cl~~ class showing deeply pigmented spots in a colorless background was related to the presence or the absence of this component. Since in the grandparent (5719A-1), in the two a_1^{m-1} carrying parent plants, and also in all plants of cultures 6452 and 6453, variegation was ~~expressed~~ exhibited, it seemed evident that the presence of this component in the chromosome complement was related to variegation and its absence to the appearance of the pale phenotype.

As stated earlier, none of the kernels on the ~~xxxxxxxxxx~~ ears derived from test crosses with the a_1^{m-1} carrying plants grown in the summer of 1951 had been examined carefully up to this time. When it was realized that the ratio of kernel types appearing on the ears of plants entered in table 3 ~~indicated~~ was related to ~~xxxxxxxxxxx~~ segregation of an independently located controlling element ~~in the chromosome complement associated with control of a_1^{m-1} behavior~~, the kernels on ears produced by test crosses of plants in the parent culture, 6046,

6047, were then examined. More direct evidence of the presence of ~~this~~ such an element was obtained, ~~for~~ and it was found to be linked in some plants ~~to the genetic~~ with the marker, y, in chromosome 6 (Y, yellow starch in endosperm; y, recessive allele, white starch in endosperm). The plants in ~~this~~ culture 6047, were derived from kernels on ~~an~~ the ear produced by the cross of ~~a plant~~ to homozygous for a_1, Sh_2 and y, ~~by~~ plant 5719A-1, which was a_1^{m-1} Sh_2/a_1 sh_2, Y/y, ~~entered in~~ C of table 2. Some kernels ~~in the~~ variegated ~~Y class~~ with yellow (Y) starch on this ear ~~were~~ sown in the summer of 1951 under A of culture 6047. Others that were variegated but had white starch (y) ~~were~~ sown under B and C of culture 6047. ~~In addition~~, variegated kernels that were Y, ~~were~~ also selected from the ear produced by self-pollination of plant 5719A-1/and shown under B and C of culture 6046. (A, table 2) All plants in cultures 6046 and 6047 received some type of test by means of crosses to plants of known constitution, ~~s~~ by means of both types of test. by self-pollination, or ~~both~~. The kernel ~~types~~ phenotypes on ears obtained by crosses of these plants to plants that were homozygous for a_1, sh_2 and y are given in table 4. All plants entered in column 1 in this table were a_1^{m-1} Sh_2/a_1 Sh_2. Those in Part I were Y/y whereas those in Part II were y/y.

In Part I of table 4, a ratio of 1 pale to 1 variegated kernel among those showing pigment ~~was exhibited among the kernels~~ appeared on ears produced from crosses conducted with plants 6046B-1 and 6047A-3.

Linkage of the pale phenotype with Y and the variegated phenotype with y is clearly expressed by the test with plant 6046B-1. In plant 6047A-3, these linkage relations were reversed; the pale phenotype was linked with y and the variegated phenotype with Y. The ratio of kernel types obtained from tests of the other plants in Part I of table 4 indicated that each carried more than one ~~of this independent~~ controlling element, ~~in the a, m-1 system.~~ In plants 6046B-3 and 6046C-2, ~~linkage of~~ one of the ~~elements~~ was linked with Y, ~~is evident~~ but in plant 6047A-1, no evidence of such linkage ~~is shown~~ was given.

The plants in Part II of table 4 were all y/y in constitution and thus no linkage relationships could be shown by them. Nevertheless, the evidence suggested the presence of more than one independently located controlling element in all plants except 6047C-3. The plants in culture 6452 (table 3) originated from the y class of variegated kernels produced on the ear by the cross of plant 6047A-3 entered in table 4. According to the ratio of kernel types on the ear, ~~data of table 4~~, this plant had only one independent controlling element and it was located in the Y carrying chromosome. The plants in culture 6453 originated from the variegated class of kernels produced on the ear from the test cross of plant 6047C-4, table 4. The ratio of kernel types ~~on its test cross~~ thus ear suggested the presence in this plant of two ~~of the independent~~ controlling elements, each occupying a different location in

the chromosome complement. Selection ~~of the y class of~~ variegated kernels that were used was made in order that the color of the anthocyanin pigment in the aleurone layer in kernels on ears of plants derived from them would be expressed on a white rather than a deep-yellow background. As stated earlier, the only purpose of this test had been to determine crossing over between a_1^{m-1} and Sh_2.

From the ratio of kernel types given in table 4, and from those obtained from self-pollination and from the test cross of plant 5719A-1, (A and C of table 2), it would appear that plant 5719A-1 had more than one ~~independently located~~ controlling element, ~~and that~~ one of them, however, occupied a position in its y bearing chromosome 6.

As mentioned above, the plants in culture 6452 (table 3) arose from variegated kernels on the ear of plant 6047A-3, which had one controlling element. Thus, ~~whose kernel types are entered in table 4. These~~ plants in culture 6452 would be expected to have only one such ~~independently located~~ controlling element. This appeared to be true for all plants except plant number 3. The plants in culture 6453, table 3, arose from variegated kernels on the ear produced by plant 6047C-3, which had two controlling elements, ~~whose kernel types are entered in table 4.~~ independently located in the chromosome complement. The plants in culture 6453 would therefore be expected to have either one or two ~~of the independently located~~ such controlling element. As the data in table 3 suggest, ~~indicates,~~ ~~xxxxxxxxx~~ seven of the nine plants in this culture had one such element

and two plants, ~~but~~ the remaining 2, plant 3 and plant 9, had more than one ~~such element~~ and the ratio of kernel types on ears produced by different parts of plant 9 suggested that the number of this element was not the same in all parts of ~~this plant~~. It was suspected from this that the ~~independently located~~ controlling element could undergo transposition in somatic cells and that this mechanism was responsible for the observed ~~deviations from expected ratios~~ aberrancies.

3. Initial evidence ~~suggesting~~ of the primary mode of operation of the independently located controlling element in the a_1^{m-1} system, the Suppressor-mutator.

The mode of control of a_1^{m-1} action induced by the independently located element of the system, whose discovery was outlined above, was not appreciated until the results of tests made in the summer of 1953 were analysed. From them, a reasonable interpretation could be drawn, ~~of its mode of operation~~. With this interpretation in mind, all test cross ears that had been produced in the summer of 1951 were then carefully examined for the first time. The evidence obtained from them ~~fitted nicely~~ conformed with this interpretation. ~~This suggested the types of~~ It was possible, then, to devise precise tests of the validity of this interpretation ~~of this that should be made~~ and these were conducted in the summer of 1954. Before these tests are considered, the evidence ~~leading to an appreciation of~~ suggesting the mode of operation of the independently located contolling element will be outlined, ~~briefly~~.

deriving from examinations of kernel types on test cross ears produced during the summer of 1951

Due to unforseen conditions, the total planting during the summer of 1953 had to be restricted. Space was available for only a fraction of the number of plants usually grown in the summer season. For this reason, study of a_1^{m-1} was ~~xxxxxxxx~~ very much limited, preverence being given to other projects then under investigation. ~~The reason that~~ The decision ~~was made~~ to limit study of a_1^{m-1} was dictated by the fact that up to this time, many of the test cross ears ~~xxxxxxxx obtained~~ produced from tests conducted in the summer of 1951 had not yet been examined and ~~xxxxx~~ little was ~~really~~ known of the behavior of a_1^{m-1}. It was decided, then, to ~~limit~~ confine its study to an examination of the number and positions of the independently located element in the system in the progeny obtained from plant 6047C-2 ~~of~~ (table 4), and to the ~~plants~~ of kernels derived from the pale class in the progeny of plant 6046C-4. This latter plant had been derived from a variegated kernel on the self-pollinated ear of plant 5719A-1 (A_1 table 2). The constitution of plant 6047C-4 was a_1^{m-1} Sh_2/a_1^{m-1} Sh_2, Y/y, pr/pr/. The ear from which the pale kernels were selected had been produced by self-pollination of this plant. On this ear ~~It gave rise to~~ 406 kernels, ~~with the following phenotypes:~~ Among them there were 35 uniformly pale colored kernels of which 33 were Y and 2 were y and to 371 kernels that had deeply-pigmented spots in a colorless background and 267 of these were Y and 104 were y. Twenty pale kernels that were selected from this ear and plants grown from them under during the summer of 1953

culture number 6628. ~~The decision to select~~ pale kernels were selected ~~from this ear~~ ~~was~~ because the plant which bore this ear was homozygous for a_1^{m-1}, and thus both ~~each~~ chromosomes 3 in a plant derived from a pale kernel should carry a derivitave of a_1^{m-1}. It was desired to examine the ~~behavior~~ ~~of each of~~ phenotypic products of these derivatives in each chromosome 3.

Therefore the plants derived from the pale kernels were tested for this by crosses with plants homozygous for a_1, sh_2 and y, and, as will be pointed out, nearly all of the kernels on the resulting ears had the pale phenotype.

Plant 6047C-2, table 4, had the constitution A_1^{m-1} Sh_2/a_1 Sh_2, pr/pr, and Y/y. An ear of this plant had been crossed by a plant that was homozygous for a_1, sh_2, y and had Pr in one chromosome 5 and pr in its homologue. The types of kernels appearing on the resulting ear, given in table 4, suggested that plant 6047C-2 had one ~~of the~~ independently located controlling element ~~of the a_1^{m-1} system~~ in the Y bearing chromosome 6 and, in addition, two others located elesewhere in the chromosome complement. Ten kernels in the variegated, Y, Pr class and ten other in the variegated, y, Pr class were selected and sown in the summer of 1953 under culture numbers 6629A and B. Nearly all the ears produced by the plants derived from these kernels were used in crosses with plants that were homozygous for a_1, sh_2 and y. Two stocks were ~~used~~ employed for this, but their constitution with respect to the Pr alleles was not then known. Tests conducted with them indicated that one stock was Pr/Pr and the other pr/pr. The need for knowning the constitution of the tester stocks with respect to the Pr alleles

will be evident shortly. In addition, a few ears of plants in culture 6629 were either self-pollinated or sib crossed.

The phenotypes of kernels on the ears of plants in culture 6629A, produced by the test cross, are entered in table 5. Again, it will be noted that there is very close linkage between the kernels carrying the Sh_2 marker and those exhibiting pigment. The position of the ear on the plant is indicated. It will be noted that on all the ears of plants A-2, A-5, and A-8, and on the first ear of the main stalk of plant A-7, there was a ratio of 1 pale kernel to 1 variegated kernel among the anthocyanin pigment bearing classes, and that the pale phenotype was linked with Y whereas the variegated phenotype was linked with y. To facilitate ready appreciation of this, the phenotypes of the pigment bearing classes of kernels on these ears are entered in table 6. The ratio of kernel types entered in table 6 / On the remaining ears of table 5, exhibited other ratios, and for a more ready appreciation of this, the numbers of kernels in the pale and variegated classes are entered in table 7. It would appear from these ratios that more than one independently located controlling element was present in plants A-2, A-5 and A-8. Linkage of the pale with Y was given by the ratios this class of kernels on the ear obtained from plant A-8 but on none of the other ears was there good evidence of this. The ratio of kernel types on the ear produced by the tiller of plant A-7 was very aberrant, only 4

variegated kernels appeared among the total of 112 that had anthocyanin in them. From the ratios of Sh_2 to sh_2 on this ear, it was clear that there was no deficiency in transmission of the a_1^{m-1} bearing chromosome.

The types of kernels appearing on the ears produced by plants in B of culture 6629, following the test cross with plants that were homozygous for a_1, sh_2 and y, are entered in table 8. An approximate 1 : 1 ratio of pale to variegated kernels was expressed on the ears produced by plants B-1, B-3, B-4, B-7, and B-10 and on the first ear of B-5. On the remaining 10 ears, whose kernel types are entered in this table, other ratios of these two classes of kernels were expressed. On several ears, a class of kernels appeared that exhibited a marked alteration in pattern of variegation. The number of such kernels is indicated by an asterik. These kernels were colorless except for 1, 2, or several very small dots that exhibited the A_1 phenotype. It will be noted, also, that on those ears in which they appeared in appreciable numbers, likewise exhibited a marked increase in the proportion of the colorless Sh_2 class of kernels. As will be shown later, these totally colorless, Sh_2 kernels, with rare exceptions, belong with the variegated class of kernels. On a few of the ears whose kernel types are entered in table 5, one or several kernels exhibiting only one or a very few small dots of the A_1-type pigment likewise

were present but because the table already is complicated, already had many entries, they were not indicated in it, and a description mention of them was deferred until this time. (It will be mentioned may be stated now, however, that study of the progeny derived from those kernels with these very decided modification of variegation expression revealed the Spm-w state of the Spm element and this will be considered in detail in section 00.) It should be mentioned here also that the kernels exhibiting the modified variegation pattern either a very restricted number of sectors or sometimes no pigmented sectors at all were not always randomly distributed over the ear. In some ears, they were adjacently aligned, forming a cluster on the ear.

In addition to the test crosses conducted with plants in culture 6629, already described, an ear of a tiller of plant 6629A-2 and 6629A-4 had been was self-pollinated and pollen collected from this tiller had been of plant 6629A-4 was placed on the silks of a tiller ear of plant 6629B-2 and 6629B-6. The ratio of kernel types on the resulting ears need not be given here as they add little to the information already gained given. However, the phenotypes of some kernels among them served to illucidate the mode of operation of the independently located element and this will be discussed shortly after the test crosses with plants in culture 6628 are considered.

As mentioned earlier, page 00, the plants in culture 6628 were derived from the pale, Y, pr class of kernels on the self-pollinated ear of plant

6606C-4, which was homozygous for a_1^{m-1}, Sh_2 and pr but heterozygous for the alleles of Y (Y/y). In order to learn whether or not the derivatives of a_1^{m-1} in each chromosome 3 would express the pale phenotype and in the samme manner by each, these plants were crossed by plants homozygous for a_1, sh_2 and y. All kernels on the resulting ears were uniformly pigmented and the grade of intensity of this was the same in all of them. Some of the a_1, sh_2, y tester plants were also homozygous for Pr whereas other were homozygous for pr. From comparisons of pigment type in kernels on ears produced by use of these two types of tester plant, it was clearly apparent that with Pr present, an intense anthocyanin pigmentation appeared in the aleurone layer of the kernel, approaching close to that appearing when A_1 is present. In contrast, those that were homozygous for pr was much less than that which is produced with A_1. There could be no confusion in distinguishing a pale, pr, class from the A_1, pr class. With this in mind, attention will be given to the note entered at the base of table 5 and table 8.

Here is given the number of kernels that expressed the full A_1 phenotype in the pr class. Those of this type in the Pr class could not always be distinguished so readily and therefore they are included in the pale classes in these tables. The frequency of appearance of kernels with an A_1 phenotype is so low that inclusions of a few of them in the pale

kernel types

classes does not invalidate the ~~significance of the given ratios~~ deductions drawn from the distributions of ~~these~~ tables. (In this regard, it may be mentioned here that in subsequent study of a_1^{m-1} it was often necessary to use another state of a_1^{m-1} that gives rise to kernels exhibiting a new pale phenotype in both the purple (Pr) and red (pr) classes, ~~that is readily distinguished from the purple and red classes produced by A_1.~~)

In examining the kernels on the ears derived from cross of plants 6629B-2 and 6629B-6, which were y/y, by plant 6629A-4, which was Y/y, some ~~appeared~~ kernels were found that had ~~several~~ areas exhibiting the pale phenotype. In addition to the deeply-pigmented spots In the Pr class, the pigment in these areas was quite intense. In the pr class, in contrast, the pigment intensity in the ~~areas~~ was low. In fact, with respect to pigment ~~intensity~~, the pale areas exhibited similar intensities ~~of pigment~~ as those expressed by the pale kernels in the Pr and pr classes ~~of kernels~~ on ears entered in tables 5 and 8. In plant 6629A-4, one independently located controlling element was present and it was carried in its y-bearing chromosome. As a consequence of ~~By means~~ of crossing over, some of the Y carrying gametes produced by this plant should have this element, and in the crosses with plants in 6629B, some kernels on the resulting ear should ~~have only one of this element and~~ should be present that the element is carried in the Y bearing chromosome.

Among the Y class of variegated kernels on this ear, several kernels were found that exhibited white starch immediately benigh a pale area, and the correspondence in their borders was exact. There seemed little doubt that the appearacne of the pale area was associated with loss of the segment of the Y-bearing chromosome 6 that removed both Y and the independently located controlling element. In other words, the pale areas appearing in the variegated kernels arose removal (or inactivation seen later) of the independently located controlling element.

No attempt was made to cross plants of culture 6629 to those of culture 6628, which were homozygous for a derivative of a_1^{m-1} and for pr, and y. Similar types of cross had been made in the summer of 1951 even though the ears derived from them had not been examined. However, on one ear of a plant of culture 6628, which had been used in a cross with a plant homozygous for a_1, sh_2, y, and pr, a single variegated kernel was present. It was a contaminant in that it had Pr and Y instead of pr and y. It Obviously was derived from functioning of a stray pollen grain produced by a plant in culture 6629A. These plants were growing in the next row and were immediately adjacent to those in culture 6628. Since this kernel showed spots of deep pigment in a colorless background as well as a few pale areas, it seemed evident that the independently located element

in the a_1^{m-1} system had a duel function. In its absence, a_1^{m-1} functioned in the production of anthocyanin pigment, although in a manner not ~~totally~~ comparable to A_1. In the presence of this element, on the other hand, all evidence of this ~~function~~ was suppressed. In its absence, ~~the mode of functioning of~~ a_1^{m-1} behaved as a stable allele of A_1. ~~was constant, no change in this occurred.~~ In its presence, on the other hand, return to ~~the~~ an A_1-type capacity of action ~~of functional expression arose~~ was initiated and A_1-like pigment was expressed in its proper; in some cells, ~~and~~ the time during development when this change in mode of action occurred ~~in a cell~~, and the number of cells in which this took place, was genetically controlled. Careful examination of ears produced during the summer of 1951 was then immediately undertaken with the newly gained insight into the system controlling gene action at a_1^{m-1}. ~~Enough insight into~~ Sufficient appreciation of the mechanism associated with control of gene action at a_1^{m-1} had been gained to allow meaningful interpretations to be drawn from ~~them, which had not been true up to this time~~ these examinations. Up to this time, this had not been true. These examinations substantiated the interpretation of the mode of operation of the independent element and therefore this element was given the designation Suppressor-mutator and symbolized as Spm. Before considering the evidence obtained from these examinations, a resume will be given of the results so far obtained, the interpretations drawn from them, and the problems they posed.

Studies outlined in this and the previous section were conducted with the progeny of oneplant, that of plant 5719A-1. In this plant, the expression of ~~the~~ a_1^{m-1} l~~ocus~~, carried in one of its chromosomes 3, was very different from that given by the parent plant having a_1^{m-1}. As stated in ~~xxxx~~ section 3 of this report, ~~it~~ was considered ~~to have~~ to be present in plant 5719A-1 an altered state of a_1^{m-1}. In plants having this state and also Spm, small streaks with ~~or~~ deep-anthocyanin pigmentation appear in a non-pigmented background although occasionally, a large area exhibiting this phenotype may appear. In the kernel, small ~~xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx~~ deeply pigmented spots appear in a non-pigmented background (photo.) although, here also, ~~an~~casionally, a large area of this type appears. The ~~described~~ same phenotypes ~~were~~ exhibited by the kernel that gave rise to plant 5719A-1, by ~~the~~ plant itself ~~and it also~~ appeared in the majority of variegated plants ~~and~~ kernels in the progeny of ~~it~~ plant 5719A-1 that ~~were~~ had been carried through three generations. However, on some test cross ears produced by some of the progeny plants, a few kernels appeared that exhibited a marked deviation from this pattern. In them, the number of deeply-pigmented spots was very much reduced or none appeared. No explanation for this had yet been found. Also, on a few ears produced by the test cross, a kernel exhibiting the full A_1-type pigmentation appeared. The nature of the

change at a_1^{m-1} responsible for their appearance had not yet been determined, although it was suspected to have arisen from mutation at a locus that governs Pl type of gene action and stability in this experiment.

In the absence of Spm, the 5719A-1 state of a_1^{m-1} gave rise to plants that were uniformly pigmented but the intensity of this, and its distribution within the plant was not the same as that given by Pl. ~~differed from that expressed when A_1 is present.~~ The kernels were likewise pigmented in the absence of Spm and this pigment was uniformly distributed over the aleurone layer of the kernel. It was intense with Pr but light when the kernels produced in the absence of Spm were homozygous for pr. The phenotype appeared to be quite stable, its expression was constant in successive generations and reappeared unaltered in progeny of plants exhibiting it, if Spm was absent. ~~these plants were either self-pollinated or crossed by plants in the tester stocks that were homozygous for a_1.~~

From the ratio of pale to variegated kernels on the ears produced by the test cross of plant 5719A-1 (C, table 2), and from the ratio of these kernel types on ears produced by test crosses of its progeny (tables 3 to 8), it was concluded that plant 5719A-1 had at least three Spm elements in it and that one of them was located in its y bearing chromosome. However, the number of this element in the progeny derived from a test cross did not always conform with that expected, and the test of plant 6452-3, table 3, illustrates this. It was expected to have one Spm element but the ratio of

pale to variegated kernels deviated markedly from the expected one to one. Also, similar types of test conducted with ears on different parts of the same plant or with pollen from a plant, sometimes produced of these two classes of kernels on the resulting ears that did not agree with one another. This suggested that the Spm constitution in different parts of an individual plant were not alike. It was suspected that these differences arose from somatically occurring transposition of Spm, but no tests of this had yet been conducted.

As emphasized above, the interpretations of the Spm a_1^{m-1} system were drawn from evidence obtained only from this one isolate of a_1^{m-1}. Others that produced quite different phenotypes had been isolated. It was therefore necessary to determine to what degree the behavior of each of them would conform with the interpretation that had evolved from study of but one. Tests had been conducted with them during the summer of 1951 and when the ears produced by these tests were examined, it was realized that the interpretation would apply to them. If it were considered that each isolate of a_1^{m-1}, originally selected because of the modified variegation pattern it had exhibited, arose from some alteration that had occurred to the original a_1^{m-1}, and that each such alteration was responsible for the type of variegation pattern exhibited in the presence of Spm and

also for the type of expression given in its absence. Some of the evidence for this will now be reviewed.

14. Additional evidence supporting the interpretation of the primary ~~Substantiation of the mode of action of~~ Spm ~~and of the individuality of states of~~ a_1^{m-1}.

During the summer of 1951, plants were grown from selected kernels on ears produced by crosses (5371) of the original a_1^{m-1} carrying plant to those that were homozygous for a_1. Fifty-two plants were derived from kernels that exhibited the pattern of variegation given by this original state of a_1^{m-1} (photo.). Seven were derived from kernels whose aleurone layer was uniformly but lightly pigmented ~~, but the intensity of this was low~~. In addition, plants were grown from two kernels, each showing a ~~with very much~~ modified pattern of variegation. One had very many small ~~, deeply pigmented~~ A1 spots in a colorless background. The other had spots of pale pigmentation on a colorless ~~had many such spots and, in addition, several large, deeply pigmented areas~~ background. In addition, kernels were selected from ears produced by crosses conducted with a_1^{m-1} carrying ~~of~~ some of the plants grown in the greenhouse during the winter of 1950-51. Selections were made from ears produced either by self-pollination or by a cross with a plant homozygous for a_1. of the plant having a_1^{m-1} ~~and involving~~ plants 5700A, 5718, 5719A-1, 5719A-2, and 5720. were so selected. ~~The ratio of kernel types on ears produced by plants 5718, 5719A-1, and 5719A-2~~ Those kernels selected from these plants to be grown in the summer of 1951 are recorded below. Tests of the plants derived from them will then be ~~are~~ entered in table 2.

Correlated.

Plant 5700A, derived from a very pale colored kernel, developed intense anthocyanin pigment in stalk, leaf-sheath, and glumes. It was used in a cross with a plant homozygous for a_1. On the resulting ear, half of the kernels were very pale colored and half were colorless. It had a derivative of a_1^{m-1} that had been received from the original a_1^{m-1} plant, in one chromosome 3 and a_1 in the other. Plants were grown from some of the pale kernels on this ear.

From the self-pollinated ear of plant 5718, some of the variegated kernels were selected and plants grown from them. Plant 5720, described in section 3, had a modified state of a_1^{m-1} in one chromosome 3 and a_1 in the other. This state produced both large and small areas in plant and kernel that exhibited only low levels of pigment intensity in them. Some variegated kernels on the self-pollinated ear and on two crosses with plants homozygous for a_1 were sown in this summer of 1951.

On the self-pollinated ear of plant 5719A-1 and of plant 5719A-2 (A, table 2), and also on ears produced by cross of these plants to plants homozygous for a_1, (C, table 1), some kernels appeared that had areas of various sizes exhibiting a pale phenotype, in addition to the deeply-pigmented spots which appeared in all variegated kernels. The number of such kernels among the variegated class was low. The majority of them exhibited only

deeply-pigmented spots that were nearly always small in size. Therefore, plants were grown from each type of variegated kernel.

Table 9 is constructed to show the types of kernels that were selected from ears produced by crosses of plants grown in the greenhouse, and the culture number given to the plants derived from each selection.

Discussion will commence with tests conducted with plants derived from selected kernels on the ear produced by the cross of plant 5719A-2 to one that was homozygous for a_1. Plant 5719A-2 was a_1^{m-1}/a_1 in constitution. On the ear this cross produced, there were 24 uniformly pale colored kernels, 250 kernels exhibiting dots of deep color in a colorless background, and 259 totally colorless kernels. Among the variegated class of kernels, some showed, in addition to the deeply-pigmented dots, areas of light pigmentation, all of which had the same grade of intensity. In a few kernels, lightly pigmented areas were large, covering as much as an eighth of the area of the aleurone layer. There was, however, much variation in size of such areas within a single kernel. Some of the kernels exhibiting these areas were selected and the plants grown from them given culture number 6081A.

The frequency of appearance of different classes of kernels on the self-pollinated ears, on ears produced by crosses with plants homozygous

A, B, and C

for a_1, are given in table 10, ~~for six of the plants in culture 6081A.~~

their

The ratio of kernel types appearing on ears ~~produced by test crosses with~~ did not agree with that

~~these plants differed greatly from those~~ appearing on ears produced by

cross

similar types of test conducted with the parent plant, 5719A-2 (A, B, C,

number of

table 2). There was a marked reduction in ~~frequency of appearance of the~~

number of

variegated ~~class of~~ kernels and an equivalent increase in ~~this~~ of ~~the~~

colored kernels. in the kernels that were pale colored

pale ~~class~~. ~~In the pale class~~, pigment intensity was greater when Pr

it was absent.

was present than when ~~the kernels were homozygous for pr~~. However, the

Pr-carrying kernels in the pale class could be distinguished readily from

those that exhibited the full or near full A_1 type pigmentation. This is

with the of

in contrast to the pale, Pr class ~~xxxxxxxx~~ produced ~~by~~ state ~~5719A-1~~

derived from plant 5719A-1

a_1^{m-1} where difficulties were encountered in making this distinction, as

described in the previous section.

From of kernel types entered Concealer Spm

~~On the basis of~~ the ratios ~~given~~ in table 10, the ~~suspected~~ constitution

in the tested part of the was assumed to be that which also to

~~of those~~ plants ~~with respect to Spm~~ is given in the last column. ~~Tests~~

constitution appeared to be the same in all tested parts of any one plant, except

~~conducted with different parts of a single plant agreed with the~~ another

that which produced the ear on the main stalk of plant A-5.

~~in this respect~~. ~~From this~~, it is ~~concluded~~ assumed, then, that plants A-2, A-3, A-4, A-6

parts of plant

and A-8 had one Spm, whereas plant A-5 had two Spm elements located at

each

a different positions in the chromosome complement.

Nearly all of the variegated kernels on ears produced by use of pollen of ~~1 Spm carrying~~ having one Spm plants on silks of plants homozygous for a_1 (C, table 10) had, in addition to dots of A_1-type pigment, areas of various sizes exhibiting the same grade of pale pigment that is present in the pale class of kernels. In other words, these kernels were similar in phenotype to the kernel from which each plant arose. ~~Some kernels of this type likewise appeared among the variegated class on the ear produced from use of pollen from plant A-5.~~ by kernels exhibiting dots of A_1 on a colorless background Among the ~~variegated class of kernels~~ produced by the reciprocal cross, B, table 10, ~~those exhibiting~~ pale areas ~~that were of various sizes were rare~~ were either absent or, if present, were few in number and small in size. ~~Pale areas were present in some kernels but they were small in size and few in number~~. It was learned in the previous section that such pale areas could be expected to appear if, for some reason, the Spm element (s) in them is lost from an endosperm cell during development of the kernel (or inactivation, see section - part -) If one Spm element is present, loss of it from some cells at various stages in development would result in the appearance of pale areas of various sizes. If two Spm elements were present, simultaneous loss of each or successive losses of them would be required in order that a pale area would appear. Because the female parent contributes two identical nuclei to the endosperm and the male parent only one, the initial Spm number in the endosperms of variegated kernels,

entered in B of table 10, would be at least two whereas the initial Spm number in the majority of those produced by the cross of plants of A-2, A-4, and A-8 in C of this table would be but one. It was obvious from observations of kernel types derived from all crosses entered in table 10 that a relation existed between the number of Spm elements in a kernel and the frequency of appearance and the size range of pale areas in it. The Spm elements was being subjected to some type of modification but the nature of this was not discovered until later.

From the same ear that produced the kernels from which the plants in 6081A were derived, another variegated kernel was selected. It exhibited only the deeply-pigmented dots. No pale areas were present. It had been selected for reasons that do not now concern us. Plant 6081B was grown from it. The types of kernels produced by self-pollination and reciprocal crosses with plants homozygous for a_1, are entered in table 11. The ratio of kernel types on these ears suggest that this plant had more than two Spm elements in it and these ratios conformed with those obtained from the parent a_1^{m-1} plant, 5719A-2 (A, B, and C of table 2). It would appear, then, that selection of variegated kernels exhibiting pale areas of various sizes in addition to the dots with A_1 type pigment in them, is an effective method for selecting those kernels on an ear that have only one or occasionally 2 Spm elements in them.

A test conducted with plants derived from variegated kernels on the self-pollinated ear of plant 5719A-2 did not negate this.

From the self-pollinated ear of plant 5719A-2, variegated kernels having dots of A_1 but no pale areas were sown under culture number 6080C. There was one variegated kernel on this ear that exhibited a number of pale areas in addition to the A_1 dots. The plant grown from it was given culture number 6080B. Reciprocal crosses of this plant to plants homozygous for a_1 produced the kernel types entered in table 12. This plant proved to be homozygous for the 5719A-2 state of a_1^{m-1}. From the ratio of kernel types on these ears, it may be concluded that plant 6080B had only one Spm element in it. In contrast, among the seven plant in C of this culture that also were homozygous for this state of a_1^{m-1}, only one had a single Spm element in it, and the ratio of kernel types appearing on ears produced by self-pollination of these plants and by reciprocal crosses of them with plants homozygous for a_1, given in A to C of table 13, indicate this.

Tests conducted with some of the progeny derived from plant 5719A-1 were discussed in previous sections. It was obvious from them that plant 5719A-1 had more than one Spm element in its nuclei. Kernels derived from the self-pollinated ear of this plant were grown under culture number 6046. Two plants were grown from the pale kernel on this ear under culture number 6046A. On self-pollination of these 2 plants and in crosses to plants homozygous for a_1, all kernels on the resulting ears were uniformly pale, like that of the kernel from which each arose. On this ear, there was a small number among the variegated kernels of plant 5719A-1

that exhibited some pale areas in addition to ~~the~~ small A_1 spots, ~~with A_1-type pigment in them.~~ Three plants ~~were~~ derived from kernels of this type. Tests of ~~them~~ indicated that in two of these three plants one Spm was present, and in the third, two Spm elements were present. ~~as the~~ In contrast, ~~data in table 14 indicate~~. Again, it was noted that the variegated kernels on ears entered in C of this table were characterized by the appearance of pale areas in addition to the A_1 spots, whereas these pale areas were rarely exhibited among the variegated class of kernels in B of this table.

Variegated kernels derived from the self-pollinated ear of plant 5718 (A, table 2) were sown under culture number 6045. Although 16 kernels were sown, only 4 plants derived from them survived to maturity. Two of them were self-pollinated and crossed to plants homozygous for a_1. The type of kernels on the resulting ears are shown in A and B of table 15. The pattern of variegation produced by ~~the~~ 5718 state of a_1^{m-1} is very sharply expressed. There are small dots of the A_1 phenotype in a colorless background, as shown in photo 00. In the absence of Spm, this state gives rise to kernels that are only very faintly pigmented. Difficulty was encountered in detecting this pigment in some kernels on the ears entered in table 15, ~~that should be carrying this state of a_1^{m-1} but no Spm.~~ Unless this pigment was undoubtedly present, the kernel was placed in the colorless